

Attorney Docket No. 9310.28CT  
In re: Goudsmit et al.  
Serial No.: 09/760,085  
Filed: January 12, 2001

### IN THE CLAIMS

Please amend the claims as follows.

1-15. (canceled)

16. (previously presented) A method for separating single stranded nucleic acid from double stranded nucleic acid, comprising the steps of:

contacting a mixture comprising both single stranded nucleic acid and double stranded nucleic acid with a first liquid comprising a chaotropic agent and a nucleic acid binding solid phase, wherein the first liquid has a composition such that the double stranded nucleic acid binds to the solid phase;

separating the solid phase from a supernatant containing the single stranded nucleic acid; and

contacting the supernatant with a second nucleic acid binding solid phase, and a second liquid consisting essentially of material selected from the group consisting of:

- a) a chaotropic agent;
- b) a chaotropic agent and a chelating agent;
- c) a chaotropic agent and divalent positive ions; and
- d) a chaotropic agent, a chelating agent and divalent positive ions,

wherein the second liquid has a composition such that the resulting mixture of supernatant and second liquid allows for binding of the single stranded nucleic acid to the second solid phase.

17. (previously presented) The method according to claim 16, wherein the first liquid comprises a chaotropic agent in concentration between about 1-10M, and a chelating agent, and has a pH between about 2 and 10.

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18. (previously presented) The method according to claim 17, wherein the chelating agent is EDTA, which is present in a concentration between about 10 mM and 1 M.

19. (previously presented) The method according to claim 18, wherein the first liquid comprises at least about 100 mM EDTA and guanidinium salt as a chaotropic agent.

20. (previously presented) The method according to claim 16, wherein the chaotropic agent is guanidinium thiocyanate.

21. (previously presented) The method according to claim 20, whereby the first liquid has the constitution of a buffer prepared by dissolving about 120g guanidinium thiocyanate in about 100ml 0.2M EDTA (pH=8).

22-27. (canceled)

28. (previously presented) The method according to claim 16, wherein the solid phase is silicium based.

29. (previously presented) The method according to claim 28, wherein the solid phase is silica.

30. (previously presented) The method according to claim 29, wherein the silica is in the form of particles having a size between about 0.05 and about 500 micrometers.

31. (previously presented) The method according to claim 16, wherein the solid phase is separated from the supernatant by centrifugation.

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32-38. (canceled).

39. (previously presented) The method according to Claim 16, wherein the concentration of the divalent positive ions is the same as the concentration of the chelating agent.

40. (previously presented) The method according to Claim 16, wherein the chelating agent is EDTA and the ions are  $Mg^{2+}$  ions.

41. (previously presented) The method according to Claim 16, wherein the chaotropic agent is a guanidinium salt.

42. (canceled).

43. (currently amended) The method according to Claim ~~42~~16, wherein the second liquid has the constitution of a buffer prepared by dissolving about 120g guanidinium isothiocyanate in about 100ml 0.35M TRIS HCl (pH 6.4) and adding about 22ml 0.2 M EDTA (pH 8.0) and about 9.1g Triton X-100<sup>TM</sup> (polyethoxylated p-isooctyl-phenol), homogenizing the solution and adding  $MgCl_2$  to a final concentration of about 0.25M.

44. (canceled).

45. (New) A method for separating single stranded nucleic acid from double stranded nucleic acid, comprising the steps of:

contacting a mixture comprising both single stranded and double stranded nucleic acid with a first liquid comprising a chaotropic agent and a nucleic acid binding solid phase in the absence of material containing alcohol groups, wherein the first liquid has a composition such that the double stranded nucleic acid binds to the solid phase;

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separating the solid phase from a supernatant containing the single stranded nucleic acid; and

contacting the supernatant with a second liquid comprising a second nucleic acid binding solid phase, in the presence of material selected from the group consisting of:

- a) a chaotropic agent;
- b) a chaotropic agent and a chelating agent; and
- c) a chaotropic agent, a chelating agent and divalent positive ions,

and in the absence of material containing alcohol groups, wherein the second liquid has a composition such that the resulting mixture of supernatant and second liquid allows for binding of the single stranded nucleic acid to the second solid phase.

46. (New) The method according to claim 45, wherein the first liquid comprises a chaotropic agent in concentration between about 1M and 10M, and a chelating agent, and has a pH between about 2 and 10.

47. (New) The method according to claim 46, wherein the chelating agent is EDTA, which is present in a concentration between about 10 mM and 1M.

48. (New) The method according to claim 47, wherein the first liquid comprises at least about 100mM EDTA and guanidinium salt as a chaotropic agent.

49. (New) The method according to claim 45, wherein the chaotropic agent is guanidinium thiocyanate.

50. (New) The method according to claim 49, whereby the first liquid has the constitution of a buffer prepared by dissolving about 120g guanidinium thiocyanate in about 100 ml 0.2M EDTA (pH 8.0).

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51. (New) The method according to claim 45, wherein the solid phase is silicium based.

52. (New) The method according to claim 51, wherein the solid phase is silica.

53. (New) The method according to claim 52, wherein the silica is in the form of particles having a size between about 0.05 and about 500 micrometers.

54. (New) The method according to claim 45, wherein the solid phase is separated from the supernatant by centrifugation.

55. (New) The method according to Claim 45, wherein the concentration of the divalent positive ions is the same as the concentration of the chelating agent.

56. (New) The method according to Claim 45, wherein the chelating agent is EDTA and the ions are  $Mg^{2+}$  ions.

57. (New) The method according to Claim 45, wherein the chaotropic agent is a guanidinium salt.

58. (New) The method according to Claim 45, wherein the second liquid has the constitution of a buffer prepared by dissolving about 120g guanidinium isothiocyanate in about 100ml 0.35M TRIS HCl (pH 6.4) and adding about 22 ml 0.2 M EDTA (pH 8.0) and about 9.1g Triton X-100™ (polyethoxylated p-isooctyl-phenol), homogenizing the solution and adding  $MgCl_2$  to a final concentration of about 0.25M.